Biomaterials 53 (2015) 484–493

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Tailoring the degradation rates of thermally responsive hydrogels designed for soft tissue injection by varying the autocatalytic potential

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ARTICLE INFO

Article history:
Received 16 December 2014
Accepted 19 February 2015
Available online

Keywords:
Hydrogel
Thermally responsive material
Degradation
Heart

ABSTRACT

The ability to modulate the degradation properties of biomaterials such as thermally responsive hydrogels is desirable when exploring new therapeutic strategies that rely on the temporary presence of a placed scaffold or gel. Here we report a method of manipulating the absorption rate of a poly(N-isopropylacrylamide) ((poly(NIPAAm)) based hydrogel across a wide range (from 1 d to 5 mo) by small alterations in the composition. Relying upon the autocatalytic effect, the degradation of poly(NIPAAm-co-HEMA-co-MAPLA), (HEMA = 2-hydroxyethyl methacrylate; MAPLA = methacrylate-polylactide) was greatly accelerated by adding a fourth monomer methacrylic acid (MAA) at no more than 2 mol% to obtain poly(NIPAAm-co-HEMA-co-MAPLA-co-MAA) (pNHMMj) where j reflects the MAA molar % in the reactant mixture. MAA residue introduction decreased the pH inside the hydrogels and in surrounding buffered solutions. Accelerated degradation positively correlated with MAA content in pNHMMj polymers, putatively by the accelerated cleavage of MAPLA residues to raise the transition temperature of the polymer above body temperature. Physical properties including thermal transition behavior and initial mechanical strength did not vary significantly with MAA content. A rat hindlimb injection model generally reflected the in vitro observation that higher MAA content resulted in more rapid degradation and cellular infiltration. The strategy of tuning the degradation of thermally responsive hydrogels where degradation or solubilization is determined by their polyester components might be applied to other tissue engineering and regenerative medicine applications where designed biomaterial degradation behavior is needed.

1. Introduction

Appropriate biomaterial degradation behavior is essential for obtaining desired therapeutic outcomes in a variety of tissue engineering and regenerative medicine applications [1–3]. Biomaterial degradation theoretically should be aligned with the pace of cell infiltration and neo-tissue formation to allow the structural and functional integration of host tissue with tissue developed in the region of the implanted biomaterial. For example, rapid degradation of dermal grafts may favor a fibrotic response over a more constructive regeneration outcome [4]. Uncoordinated absorption of bone substitutes can cause mechanical mismatch and ultimately contribute to failure in load bearing [2,5]. In biodegradable arterial stent development, there is interest in assuring that the stent remains long enough to remodel the vascular wall in a stable fashion, but not much longer to avoid complications associated with a permanent foreign body in the vascular wall [6,7].

Thermally responsive hydrogels have been widely studied for their amenability to minimally invasive delivery in the realms of drug delivery, embolization therapy, cell delivery vehicles, tissue fillers and wound dressings [8–10]. More recently, intramyocardial injection therapy has been pursued using mechanically strong thermally responsive hydrogels to inhibit pathological ventricular dilatation after myocardial infarction, a major contributor to morbidity and mortality in ischemic cardiomyopathy [11–13]. As with other biomaterial applications where temporary mechanical

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http://dx.doi.org/10.1016/j.biomaterials.2015.02.100
0142-9612/

support is the objective, the degradation behavior of thermally responsive hydrogels becomes a critical design consideration.

In seeking to control the degradation rate for a thermally responsive hydrogel that would be used in soft tissue injection, several other design criteria must be considered. Basic requirements are acceptably low levels of cytotoxicity of the polymer and degradation products and adequate thermal response to allow needle-based injection and stiffening in situ. In the role of cell carrier, one would want to maintain encapsulated cell viability. When tuning the degradation rate, one would ideally not impair the thermal sensitivity of the system or substantially alter the mechanical properties in the fluid or hydrogel state.

Polyesters, widely used as biodegradable polymers in general, have been utilized as a hydrophobic component in thermally responsive hydrogels to trigger dissolution and absorption of the hydrogels upon ester cleavage [14–16]. Polyester materials degrade faster under low pH conditions due to catalyzed hydrolysis [17]. The accumulation of acidic degradation products can lead to an autocatalytic effect, accelerating the hydrolysis of ester bonds [18–20]. It was anticipated that the autocatalysis effect could be employed to tune degradation rates across wide ranges. Thus, in this study the amount of acid in a thermally responsive copolymer backbone was varied to modulate the degradation rate of a poly(N-isopropylacrylamide) based hydrogel, poly(NIPAAm-co-HEMA-co-MAPLA) (pNHM, copolymerized with N-isopropylacrylamide (NIPAAm), 2-hydroxyethyl methacrylate (HEMA) and methacrylate-polylactide (MAPLA)) [21]. The pendant hydrophobic MAPLA sidechains become acidic units upon hydrolysis, resulting in a higher transition temperature and eventual solubility of the copolymer, without backbone cleavage. Different molar ratios of methacrylic acid (MAA) were incorporated into the copolymer to obtain poly(NIPAAm-co-HEMA-co-MAPLA-co-MAA) (pNHMMj). The effect of MAA on hydrogel degradation was studied as well as its effect on thermal and mechanical behavior. The cytotoxicity of pNHMMj hydrogels and their degradation products were evaluated and an in vivo degradation study of pNHMMj hydrogels was performed in a rat hindlimb injection model.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma–Aldrich unless otherwise stated. N-isopropylacrylamide (NIPAAm) was purified by recrystallization from hexane and vacuum-dried. 2-Hydroxyethyl methacrylate (HEMA) was purified by vacuum distillation. Lactide, benzoyl peroxide (BPO), sodium methoxide ($NaOCH_3$), methacryloyl chloride, methacrylic acid (MAA) and other solvents were used as received.

2.2. Synthesis of methacrylate polylactide (MAPLA)

The synthesis of methacrylate polylactide was performed as previously described [21]. Briefly, $NaOCH_3$/methanol was added to a lactide/dichloromethane solution to synthesize polylactide (HO-PLA-OCH_3) through ring-opening polymerization. MAPLA was synthesized by dropping methacryloyl chloride into a HO-PLA-OCH_3/dichloromethane solution containing triethylamine. Dichloromethane was removed by rotary evaporation and the product was purified by flash chromatography to obtain MAPLA with yields of ~60%.

2.3. Synthesis of poly(NIPAAm-co-HEMA-co-MAPLA-co-MAA)

Poly(NIPAAm-co-HEMA-co-MAPLA-co-MAA) (pNHMMj) copolymers were synthesized from NIPAAm, HEMA, MAPLA and MAA by free radical polymerization. The feed ratios of NIPAAm, HEMA, MAPLA and MAA were 80/(10-j)/10/j, where j = 0, 0.5, 1, 2, 5, 10 (Scheme 1). Monomers (0.066 mol) were dissolved in 180 mL of 1,4-dioxane containing 0.23 g BPO. The polymerization was carried out at 75 °C for 20 h under argon protection. The copolymer was precipitated in hexane and further purified by precipitation from THF into diethyl ether and vacuum-dried, with yields of ~80%. Fluorescently labeled copolymers were synthesized using the same reaction conditions with fluorescein O-methacrylate added at a feed ratio of an additional 2%, with all of the other monomer molar ratios remaining constant and j = 0. Fluorescently labeled hydrogels used in the in vivo study were prepared by dissolving 14.25 wt% unlabeled copolymer with 0.75 wt% labeled copolymer in PBS.

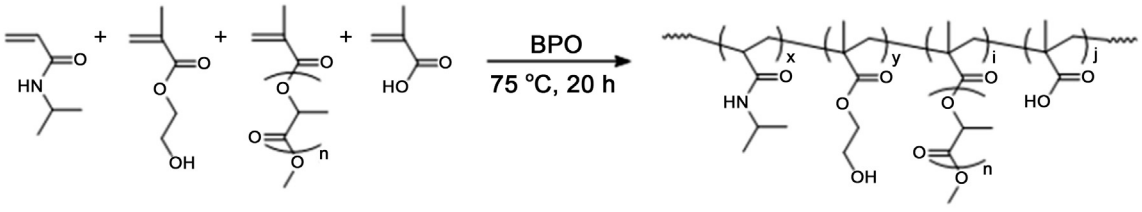
2.4. Characterization

^{1}H NMR spectra of pNHMMj were recorded with a 600 MHz Bruker spectrometer using CD_3Cl or DMSO-d6 as a solvent. Molecular weight of the copolymers was determined by gel permeation chromatography (GPC, Waters Breeze System, Waters 1515 HPLC Pump, Waters 2414 differential refractometer). The copolymers were dissolved in THF at a concentration of 1 mg/mL and the GPC analysis was performed at 35 °C. A poly(methyl methacrylate) standard kit (Fluka, ReadyCal Set Mp 500-2700000) was used for molecular weight-elution volume calibration.

Rheology studies were conducted on a TA Instruments rheometer (AR2000) to observe viscosity changes in the hydrogels during the temperature induced sol–gel transition. The polymer solutions (15 wt% in PBS) were placed between two parallel plates. With a temperature sweep from 5 to 35 °C and a heating rate of 5 °C/min, the shear storage modulus G′ and the loss modulus G″ were collected as a function of temperature at a fixed strain of 2% and a frequency of 1 Hz.

To measure the mechanical properties of the hydrogels, samples were incubated in a 37 °C water bath for 24 h to reach a stable water content, and then the solid hydrogels were cut into rectangular strips 1 mm thick, 4 mm wide, and 25 mm long and then loaded in a water bath equilibrated to 37 °C. An ElectroForce 3200 Series II (Bose, Minnesota, US) equipped with a 2.5 N load cell was utilized to record the tensile stress–strain curve immediately after the samples were taken out of the water bath.

Hydrogel degradation was quantified by mass loss measurements. Hydrogels with known initial dry masses (~60 mg) were immersed into 6 mL of PBS at 37 °C. At



MAA feed ratio (%)	Feed ratio (%)			
	NIPAAm	HEMA	MAPLA	MAA
0	80	10	10	0
0.5	80	9.5	10	0.5
1	80	9	10	1
2	80	8	10	2
5	80	5	10	5
10	80	0	10	10

Scheme 1. (a) Synthesis of poly(NIPAAm-co-HEMA-co-MAPLA-co-MAA). (b) Feed ratio of monomers.